



# Alterations in Neurons of the Brainstem Due to Administration of Inhaled Tetrahydrocannabinol: A Quantitative Histopathology Study on Rats

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**Abstract:** A Quantitative Histopathology study on rats' brainstem was used to analyze morphological alterations in the neurons and glial cells of rats that received inhaled tetrahydrocannabinol for 4, 8 and 12 weeks. Puffing of smoke was performed with the use of a Hamilton syringe delivering 100ml puffs at 20s intervals into the nose only manifold. Smoke was first pumped into a 500ml dilution chamber with the aid of a vacuum pump. The smoke was then displaced from the dilution chamber through the nose-only manifold at 300ml/min; the rats received inhaled THC at 5ml/sec for 5 minutes. After administration for varying durations a selective cell staining of the neurons and glial cells in the rat's pons, medulla and midbrain was carried out and used to study visible morphological changes in the tissues. Sections from the pons, medulla and midbrain were stained on slides for viewing under the microscope and photographed. Quantitative and qualitative histopathology study of photomicrographs was then used to analyze changes in the morphology and number of neurons. There was an increase in the ratio of neuronal cells comparing between the control and the treated groups with the pons (1:8), medulla (1:3) and the midbrain (1:5) which suggests neurogenesis and on further analysis of the slides show evidence of cell division. These findings can be of great importance in the study of neurodegenerative diseases and in understanding the influence of THC on brain.

**Keywords:** Neurons, Glial Cells, Tetrahydrocannabinol (THC), Inhalation Treatment, Pons, Medulla, Midbrain, Photomicrography, Brain Stem

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## 1. Introduction

There is growing evidence that tetrahydrocannabinol from cannabis is been used as remedies mostly as analgesics and appetite stimulant [1-3]. Among its other effects includes dilating bronchioles in the lungs and reducing intraocular (within the eye) pressure [4]. Other suggested uses are its use as possible therapy for glaucoma and asthma [5]. On the contrary so much is also been said on THC related disorders and cellular changes caused by its use [6]. The present work evaluates the changes in the histology of the cells in the

Brian stem (Pons, Medulla and Mid Brain) in rats treated for 4, 8 and 12 weeks with inhaled THC through a nose – only smoke administration model [7]. Analysis of morphological alterations has been used to achieve photomicrography and comparison of the Brain cells as seen in methods used by Sarafian et al in 2006. Michael Smith and his colleagues in 2005 [8] had reported that marijuana may grow neurons in the Brain though he explained to patients who inquire that these findings about marijuana are preliminary. Resent Bioassay studies show that inhaled THC will cause an increase in Total Brain Acetylcholine in Rats [9]. A

quantitative histopathology study on rats forebrain ischemia showed that the high dose THC-treated group showed significantly less neocortical injury, compared to either the control or low-dose THC groups [10]. The hippocampus has been reported to contain a high density of CB<sub>1</sub> receptors. The relative absence of the cannabinoid receptors from brainstem nuclei may account for the low toxicity of cannabinoids when given in overdose. The regional distribution of the CB<sub>1</sub> receptor in brain correlates only poorly with the levels of anandamide and other endocannabinoids in different brain regions [11, 12]. There has been no consistent association found between marijuana smoking and measures of airway dysfunction, the risk appears to be smaller than for tobacco, yet is important to consider when weighing the benefits and risks of smoking marijuana. [13]. Marijuana reaches the same pleasure centers in the brain that are targeted by heroin, cocaine and alcohol and recent study found a link between certain genetic markers and symptoms of marijuana addiction, suggesting that some people may have genetic predisposition to marijuana addiction [14].

## 2. Materials and Methods

### 2.1. THC (Cannabis)

Cannabis was collected from the National Drug Law Enforcement Agency (NDLEA) office Abuja; it was identified and authenticated by the National Drug Law Enforcement agency, Gudu, Abuja. Ethical approval was also received from the University of Jos ethics committee. The cannabis was burnt in a nose-only administration chamber and administered by 'Puffing' performed with the use of a Hamilton syringe delivering 100-ml puffs at 20-s intervals into the nose only manifold for a total of 5 minutes. Smoke is first pumped into a 500ml dilution chamber with the aid of a vacuum pump. (See Appendix 1 and 2) Smoke is then displaced from the dilution chamber through the nose-only manifold at 300 ml/min. That is they received Inhaled THC at approximately 5ml/sec [7].

### 2.2. Instrumentation

1. Nose- only smoke model – THC administration
2. Slides
3. Microtone
4. Automatic tissue processor
5. Electron Microscope
6. Microscope
7. Camera

### 2.3. Animals

Male albino rats weighing 180 – 260g were employed. They had free access to food and water except at the time of the experiments. There were 4 groups with five rats per group.

### 2.4. Brain Extraction, Fixing and Sectioning

Albino Rats after appropriate treatment with inhaled THC

were sacrificed and their Brains were removed excluding the cerebellum. The Brains were fixed in formal saline until when they were sectioned. Sectioning was done with the use of a microtone around the brain stem region. The sections were cut at 10nm; the areas of the Pons, Medulla and the midbrain were carefully sectioned using the microtone. The sections were tagged for slide staining.

### 2.5. Slide Preparation and Photomicrography

Slide preparation- Two different stains were employed for staining slides and they selectively stain to enable us achieve our desired result.

1. Cresyl fast violet
2. Haematoxylin and Eosin (H & E)
  - The cresyl fast violet selectively stains Neurons and cell nuclei. Viewed under the microscope and photographed. Neurons – Stain from purple to Blue; Cell Nuclei – Stains purple blue.
  - Haematoxylin and Eosin stains the tissues and its features.
  - The slides were photographed and tagged in the groups below
1. Control - Control Pons, Medulla and Midbrain
2. 4wk THC treated - 4wks THC Pons, Medulla and Midbrain
3. 8wk THC treated - 8wks THC Pons, Medulla and Midbrain
4. 12wk THC treated - 12wks THC Pons, Medulla and Midbrain

### 2.6. Quantitative Neuronal Cell Analysis

A quantitative histopathology examination of data by cell counting from all groups was statistically evaluated using Microsoft Excel. For every rat the neurons in 3 High powered fields of the stained slides were counted and the mean was used as the value for that rat. Then the mean of all the rats in each group represent Actual count for the group. The mean Neurons were reported as a ratio between the control and the treated.

## 3. Results

### 3.1. General Observation on Slides: Cresyl Fast Violet and H & E

Microscopic Slides of the Brain stem area of the Pons, Medulla and the midbrain stained with cresyl fast violet and magnified at x250 clearly show two major types of cells in the brain marked A and B.

Figure Legend:

Red arrows = Neuronal perikarya, they are Large and polygonal in shape. The Perikarya of the neurons are also seen with surrounding clear space.

Yellow arrows = are the Glial Cell.

Green arrows = oval nucleus with a prominent central nucleolus.

Black arrows= are the axon and the dendrite respectively.

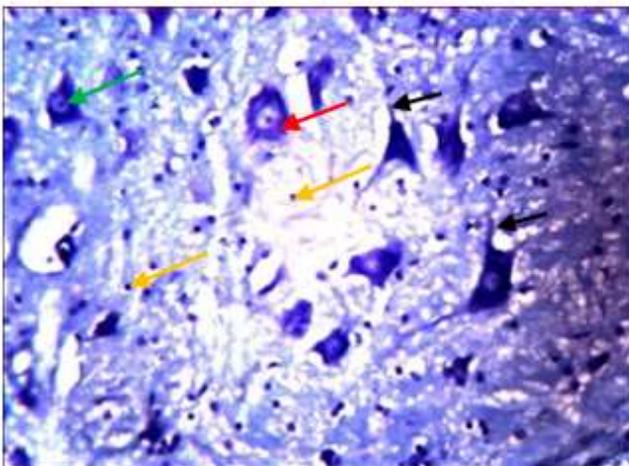
### 3.2. Observations on Glial Cells

Microglial Cells Labeled A2 is seen as spindle shaped cells. (Have a protective function). Oligodendrocytes Labeled B2 has round basophilic nuclei (dark blue in H & E stain) surrounded by a clear space around it which is also due to washed out myelin which does not stain. (Maintains the integrity of the tissues and have supportive function). Astrocytes are normally not easy to identify on H & E stains but are usually located close to blood vessels.

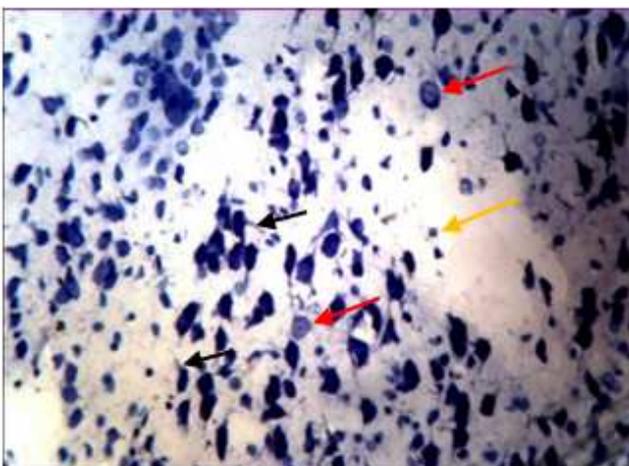
*Table 1. Illustrating the ratio of neuronal cells in each region of the brainstem.*

Region of the Brainstem	Actual Mean Counts	Estimated Ratio Of Cells
Pons	40: 325	1: 8
Medulla	82: 259	1: 3
Mid-brain	131: 611	1: 5

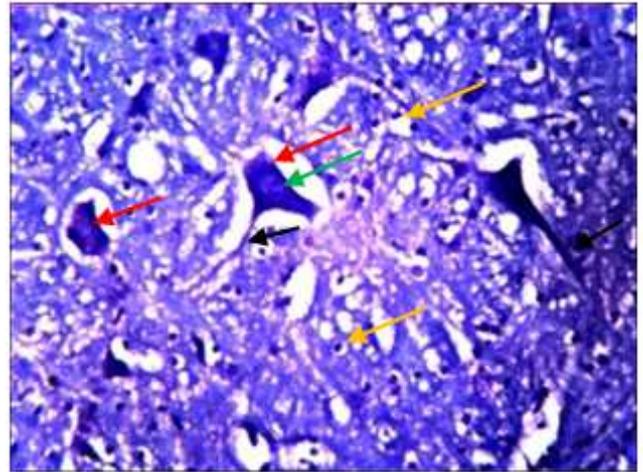
Histochemical representation of sections of the brainstems using Cresyl Fast Violet (CFV) Stain.



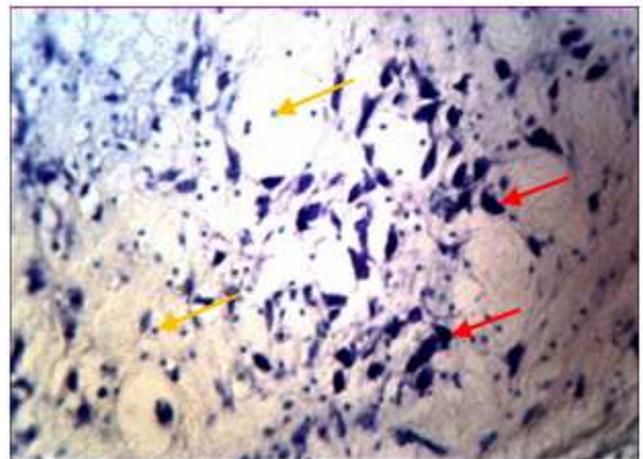
*Figure 1. Pons of Control x250.*



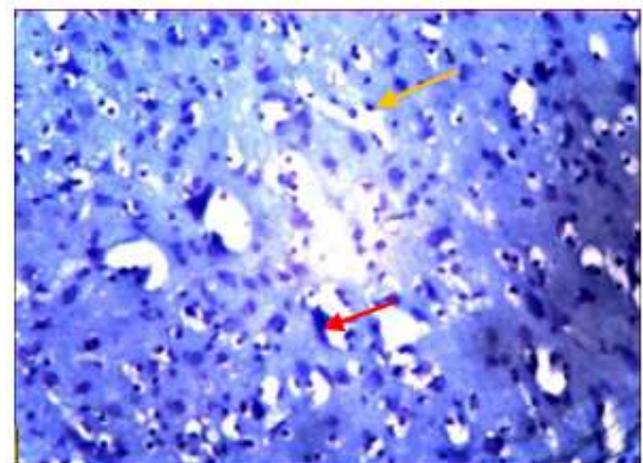
*Figure 2. Pons of Treated Rat x250.*



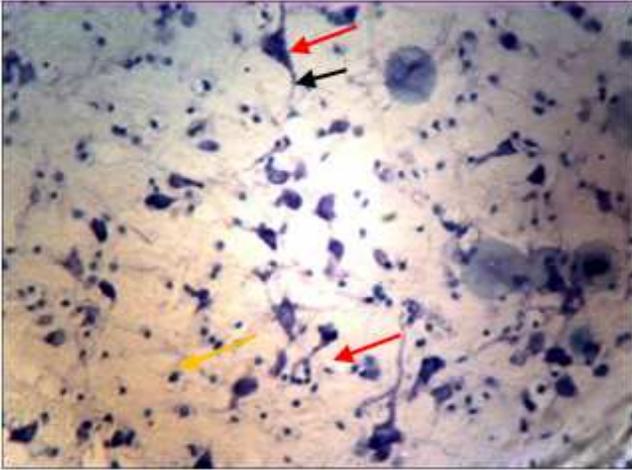
*Figure 3. Medulla of control x250.*



*Figure 4. Medulla of treated Rat x250.*



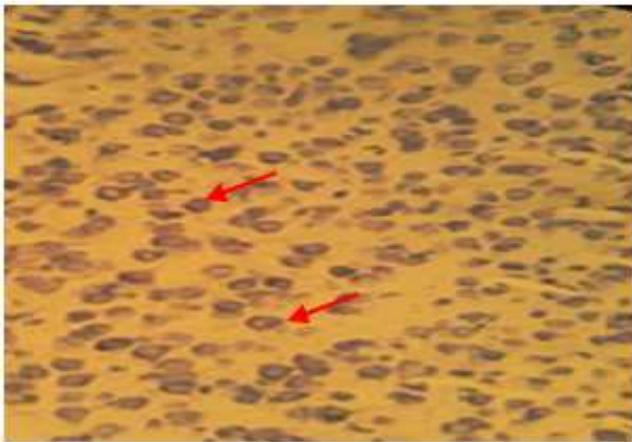
*Figure 5. Mid Brain of Control x 250.*



**Figure 6.** Mid Brain of Treated Rat x250.

#### Evidence of Cell Division

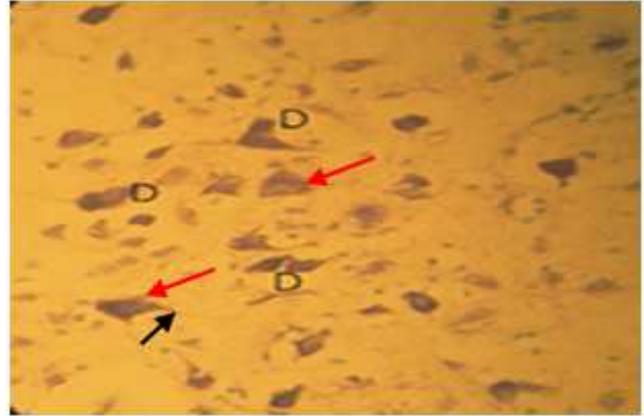
Figures 7 and 8 showing evidence of cell division marked 'D'



**Figure 7.** H & E stain of the Pons of THC-treated rats X250.



**Figure 8.** H & E stain of the Medulla of THC treated rats x250.



**Figure 9.** H & E stain of the Midbrain of THC treated rats x250.

Figures 7- 9; Show cells of the brainstem undergoing cell division. The arrows seen on Figure 10, show cell division and cells moving to opposite poles. Cells are seen to be in an advanced stage of cell division and this may be evidence of neurogenesis considering the number of cells seen on the sections studied.

## 4. Discussion

The present findings show that inhaled THC from cannabis caused an increase in number of neurons in the Pons, medulla and midbrain stained with cresyl fast violet. These were reported by counting neurons on selected brain slide stains and carrying out photomicrograph studies of the brainstem sections. Means from 3 randomly picked areas of the tissues were counted and reported as number of cells per high power field of the treated tissue, with which the mean of each group was generated and these were then compared in ratio of cells against the control tissue. The result presented only reflect the ratio of the control against the 4weeks treated as it showed the most obvious increase though the 8 and 12 weeks groups showed increase in ratio that were just similar but not more than the 4 weeks group. From figures 1 to 6 there are visible changes in the number of neurons in the control pons and the treated pons were in the ratio of 1: 8 per high power field while the neurons in the medulla were in a ratio of 1:3 per high powered field for control and treated groups respectively. Also, for the number of neurons per high power field for the midbrain there was a ratio of 1:5. All ratios reflect an increased number of neurons per high power field in the treated group though they were smaller and viewed to be immature cells. Since the treatment method was inhalation it explains why the highest increase in neurons is in the pons being the major respiratory center [7]. Neurogenesis seen on Figures 7-9 of the brainstem could be of great importance in the study of neurodegenerative diseases since proliferation of new cells due to cell division could help replace cells that have degenerated. Studies have shown increased Total Brain acetylcholine and brain activities due to THC inhalation and this results further sheds light on the increase as

increased number of neurons will cause increased neurotransmitter release and increased brain activity [9].

## 5. Conclusion

The relative absence of the cannabinoid receptors from brainstem nuclei may account for the low toxicity of cannabinoids when given in overdose or taken for long duration this may account for why much difference was not seen after 8 and 12 weeks compared with the 4 weeks. Furthermore, the increase in number of neurons is not related to the receptor site but rather the functions of the tissue as can be seen in the ratio of increase in the pons (1:8) which was the highest since it is an area of the brainstem involved in respiration and inhalation of smoke.

## Appendix 1



*Figure A1. Nose-only Smoke Inhalation Apparatus.*

## Appendix 2



*Figure A2. Rats Undergoing Nose-only Smoke Inhalation.*

## Conflict of Interest

We the authors declared that there are no conflicts of interest in this research work.

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